XENOTECH

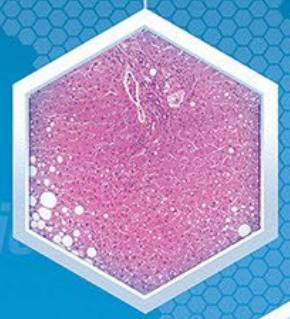
A BioIVT Company

25 Years

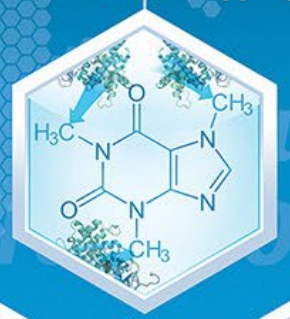
ADME | DRUG-DRUG INTERACTION | DMPK

CONTRACT RESEARCH & TEST SYSTEM EXPERTISE

Cell & Tissue-Based Products

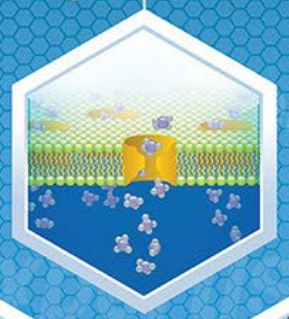


Reaction Phenotyping

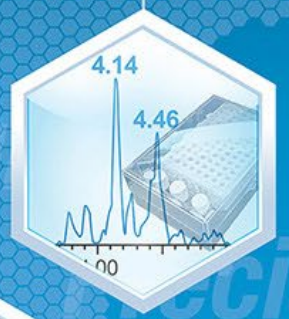


Enzyme Induction & Inhibition

Drug Transporters



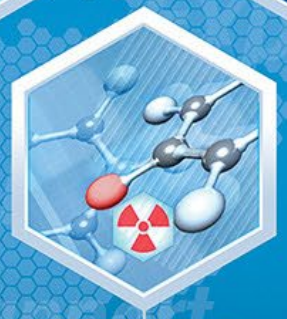
Metabolite ID & Production



Screening

Pharmacokinetics & QWBA

Radiolabeling



Bioanalytical

Role of Sulfotransferases in Drug Metabolism and Drug-Drug Interactions

Maciej Czerwiński, Ph.D.

Director, Scientific Consulting

XenoTech

Non-CYP mediated metabolism series

- **In Vitro Strategies for Evaluating Non-CYP Metabolism Pathways** by Brian Ogilvie;
- **Underprediction of Drug Clearance by Aldehyde Oxidase (AO)–Mediated Drug Metabolism: Important Considerations for In Vitro Assessment** by Pallavi Limaye;
- **Role of UDP-Glucuronosyltransferases (UGTs) in Drug Metabolism and Drug-Drug Interactions** by Maciej Czerwiński;
- Next we will cover **esterases**

Outline of today's presentation

- Introduction to sulfotransferases
- Tissue distribution
- Contributions to drug metabolism, examples of sulfated metabolites
- SULTs in the FDA Guidance and applicable test systems

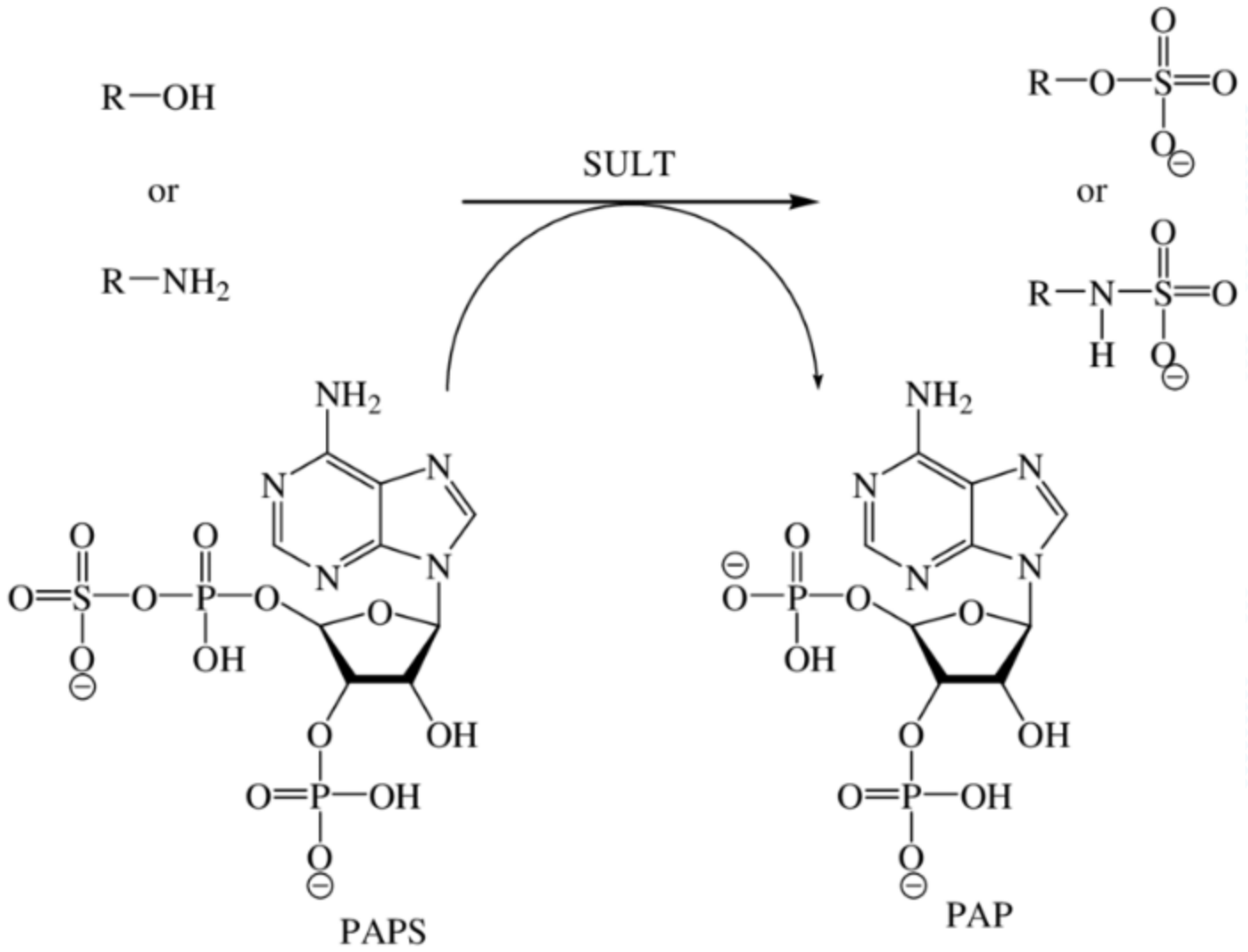
Introduction to SULT enzymes

- SULTs, expressed in a wide range of tissues, are membrane bound in the Golgi apparatus and soluble protein in the cytoplasm of liver, kidney, GI tract, lung, prostate, placenta, skin, brain, and many other tissues.

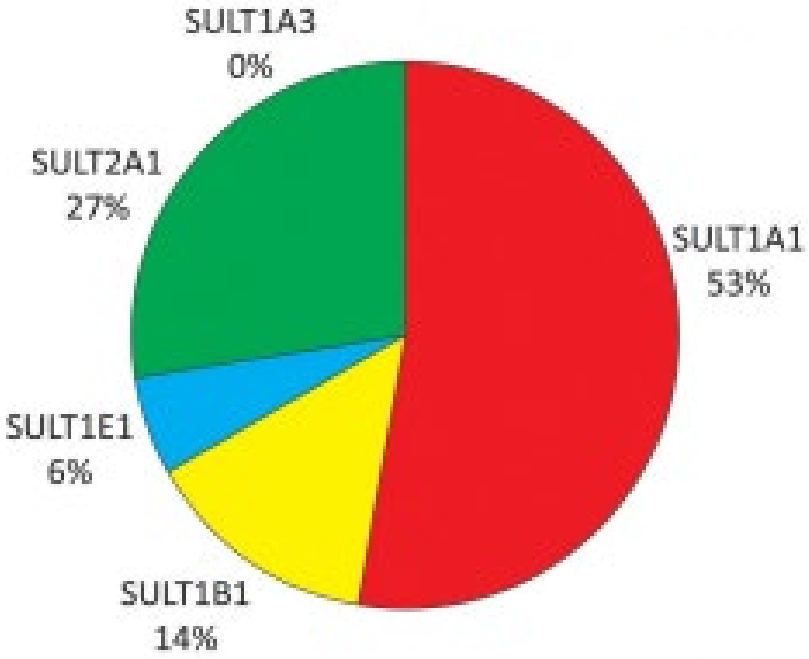
- Sulfonation reaction involves the transfer of sulfonate from the co-factor 3´-phosphoadenosine-5´-phosphosulfonate (PAPS) to the substrate. PAPS is synthesized from inorganic sulfate (SO_4^{2-}) and ATP by ATP sulfurylase followed by APS kinase (adenosine phosphosulfate kinase).

- Generally, for the cytosolic enzymes, the site of sulfonation is an electron rich (nucleophilic) O or N heteroatom. The product of the reaction is a highly water-soluble acid ester.

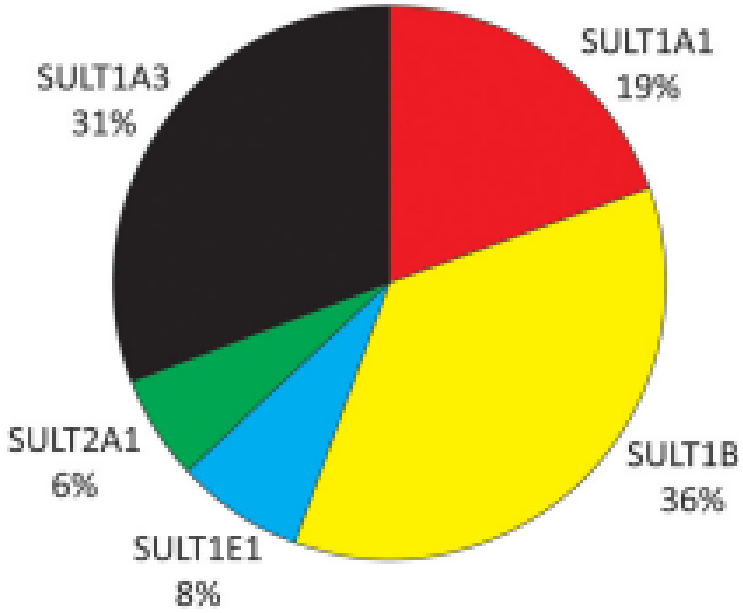
Sulfonation reaction



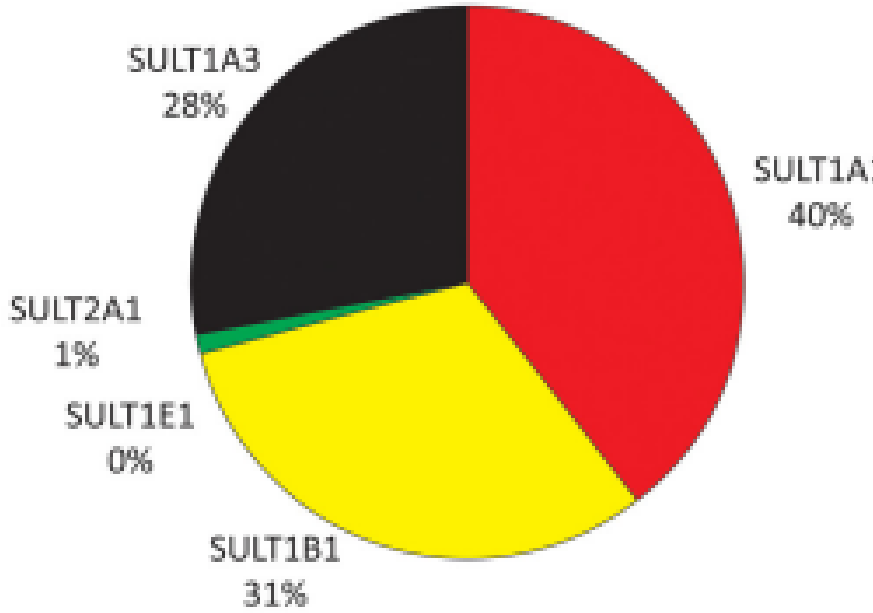
Tissues-specific expression of human sulfotransferases



Liver



Small intestine



Kidney

Riches et al., *DMD* 37:2255–2261, 2009

Role of SULTs in homeostasis of endogenous substrates

- Sulfotransferases play important roles in the sulfonation of endogenous molecules such as steroid hormones and neurotransmitters, in addition to the metabolism of xenobiotic molecules such as drugs, environmental chemicals and natural products.
- In the context of human health, it is necessary to consider the levels of expression of SULT enzymes in tissues involved in drug disposition (liver, intestine, lung, kidney, blood) or in metabolism of endogenous chemicals (gonads, adrenals, brain).

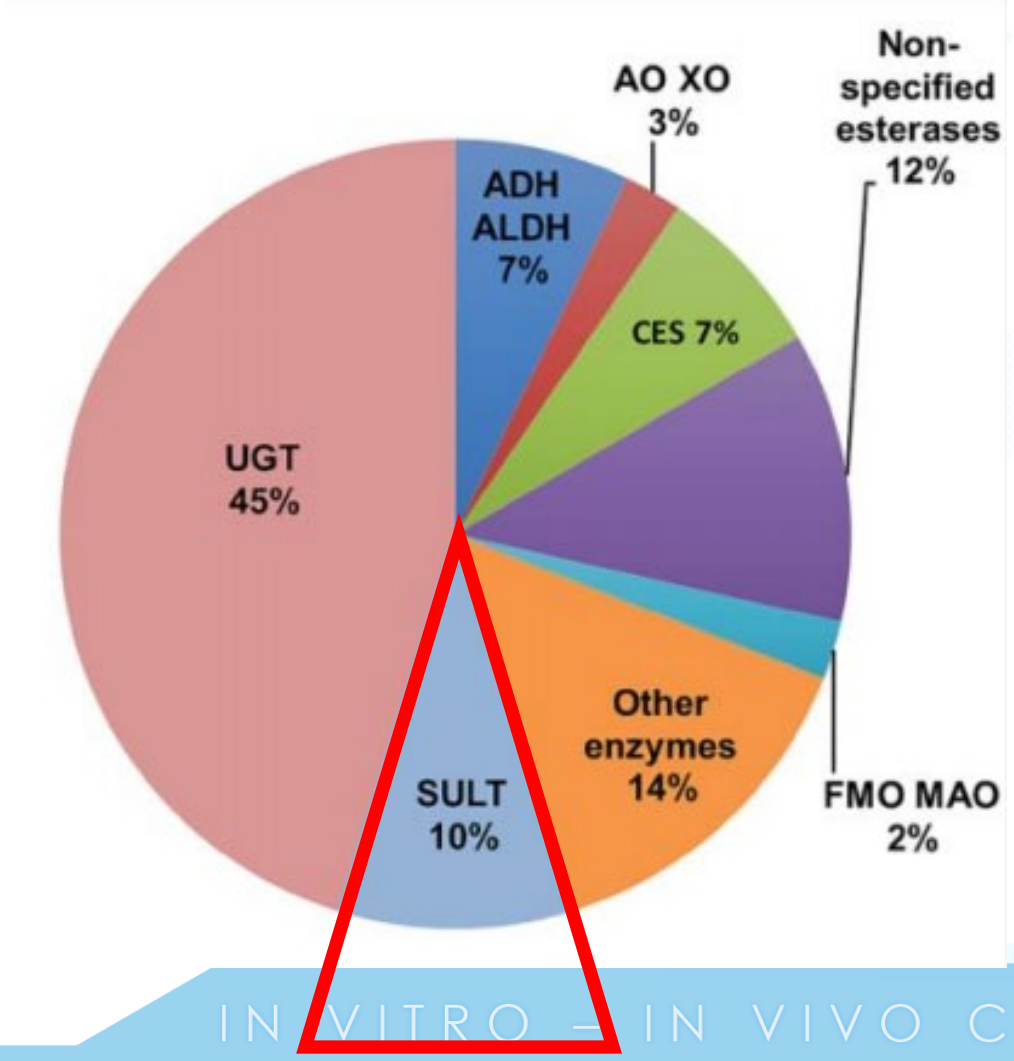
Adrenal gland	Thyroid gland	Testis	Ovaries	Endometrium	Placenta	Brain
1A1	1C2	6B1	1C4	1A1	1A1	1A1 – 4
1E1	1C3		2A1	1E1	2B1	2A1
2A1						4A1
2B1						

Parkinson et al.; *Casarett & Doull's Toxicology: The Basic Science of Poisons*, 2018

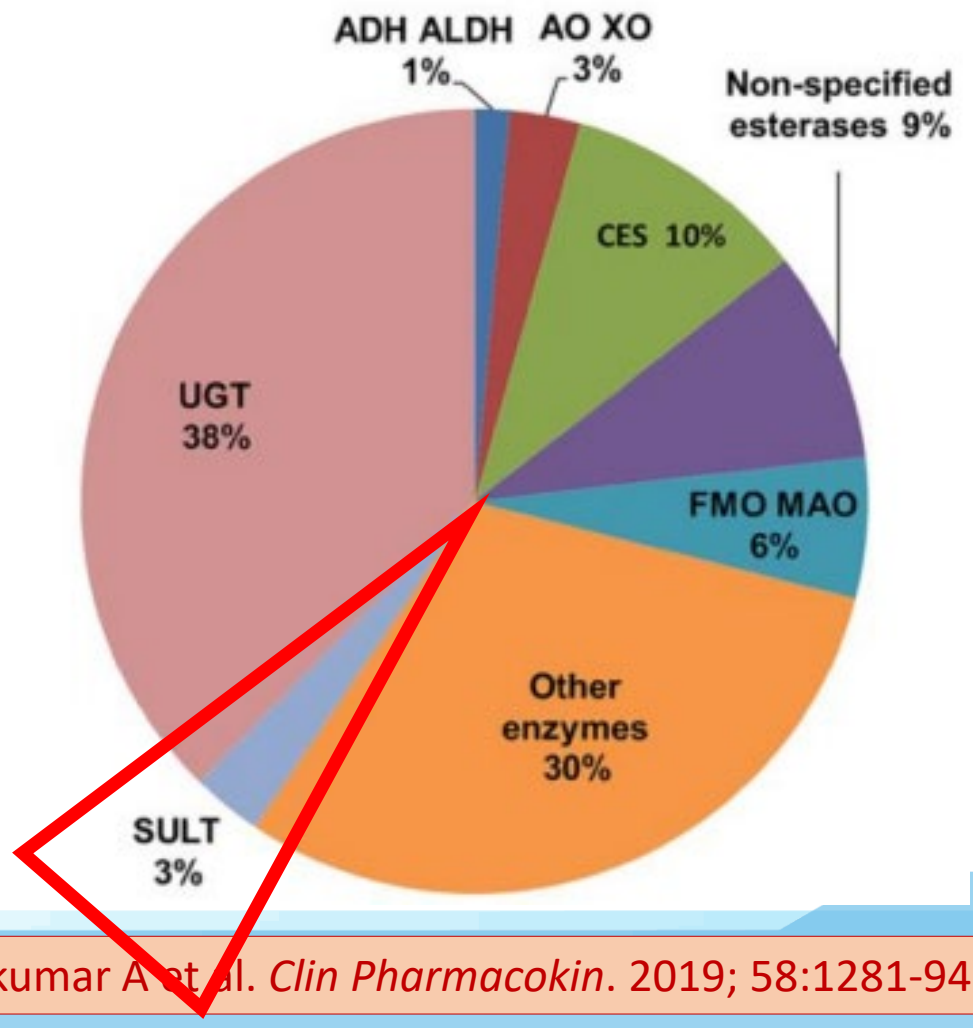
Drug Metab Rev, 2013; 45(4): 401–414

Sulfonated metabolites of drugs

Most prescribed drugs

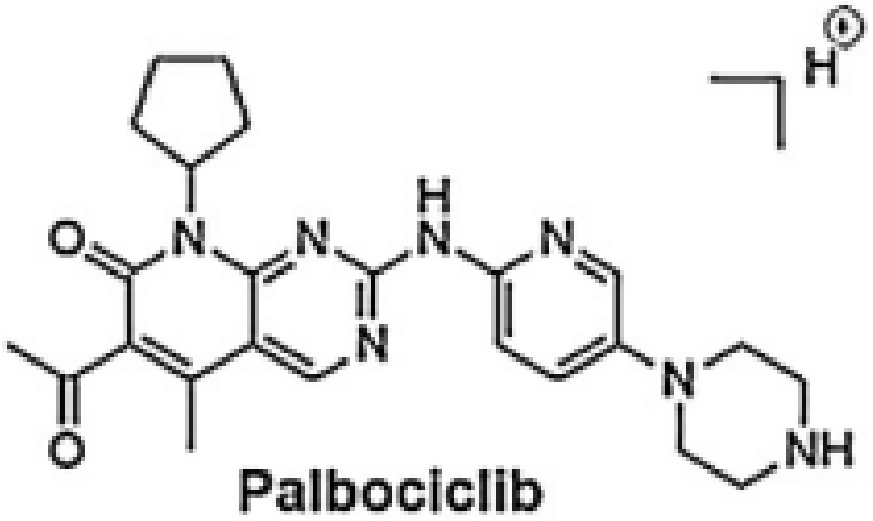


FDA approved drugs 2005 - 2016

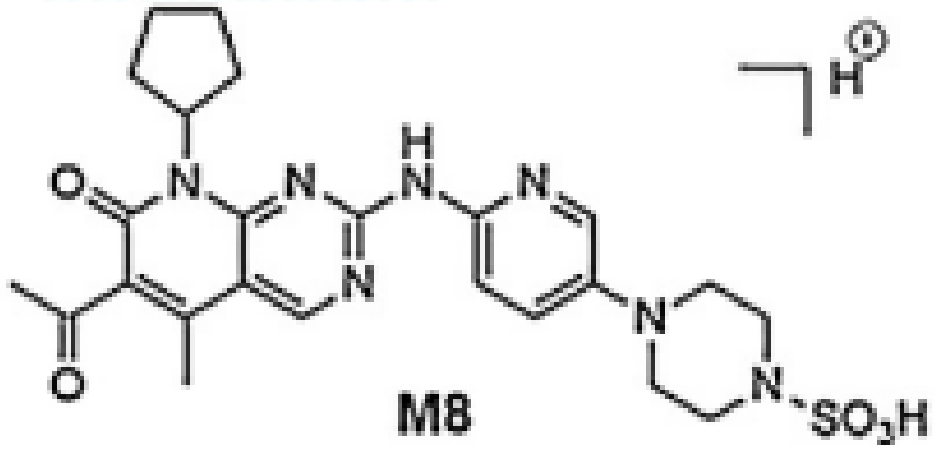


Saravanakumar A et al. *Clin Pharmacokin*. 2019; 58:1281-94

Sulfonated metabolite of palbociclib



14 primary metabolites of palbociclib are products of hydroxylation, oxidation, N-oxidation, N-dealkylation, carboxylation, carbonylation, acetylation and sulfation

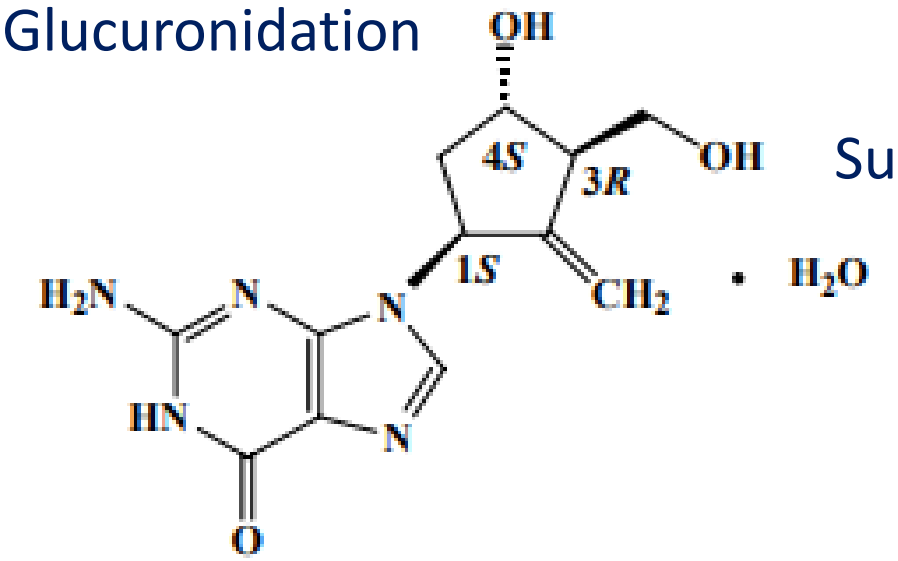


Major enzymes metabolizing this drug are CYP3A4 and SULT2A1, the second most abundant SULT in human liver. The M8 is sulfonated at the pyrazine ring

Chavan et al. *Journal of Pharmaceutical and Biomedical Analysis* 157 (2018)

Sulfonated metabolite of entecavir

Glucuronidation

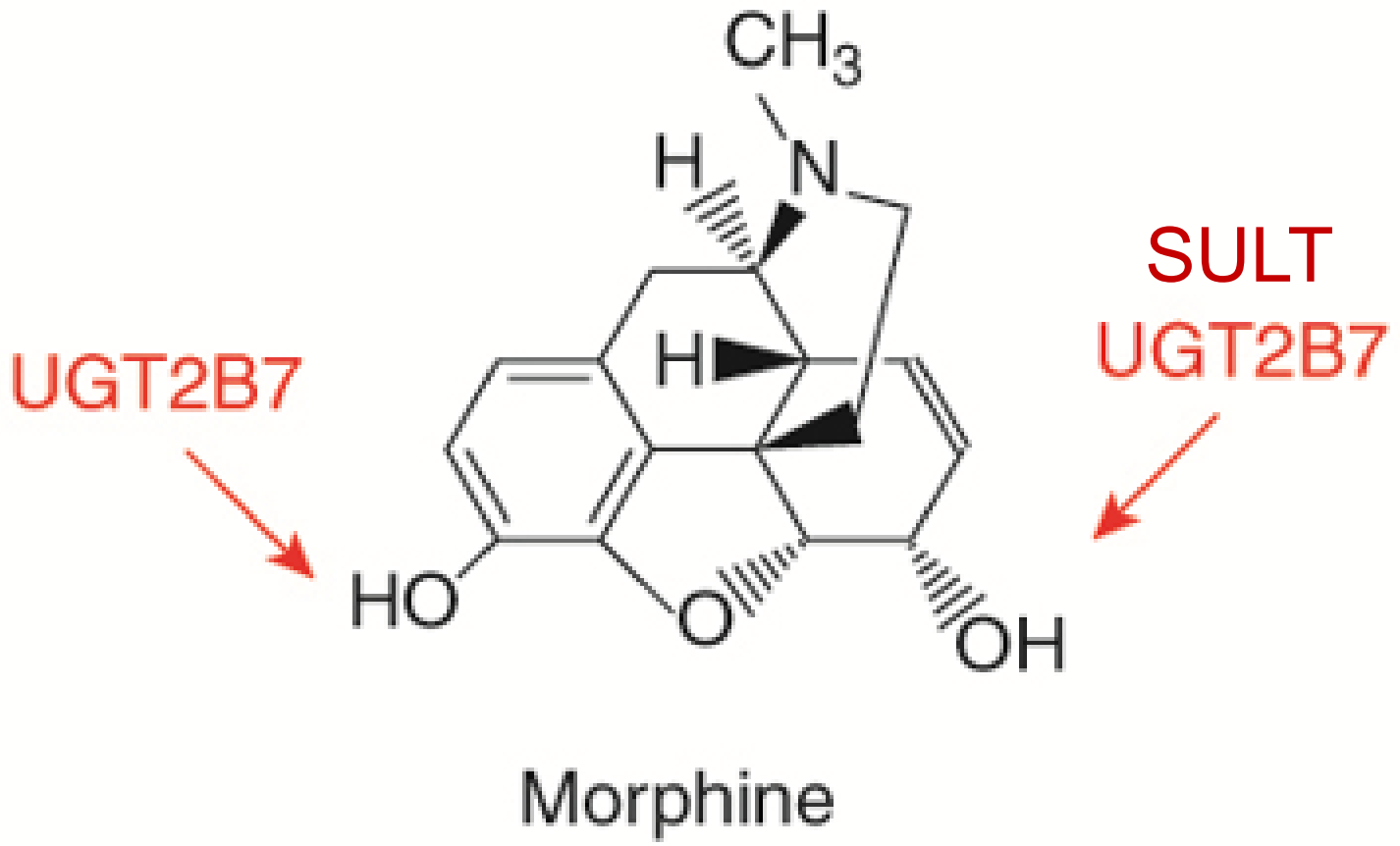


Sulfonation

Entecavir is a guanosine nucleoside analogue with selective activity against hepatitis B virus. Only a very small fraction of this drug is metabolized. No Phase I metabolites are found in vitro or in vivo. In vivo there are four major metabolites: three glucuronides and one sulfate. The sulfate was not detected in vitro and was about 15.2 % of the dose in vivo; detected only in feces.

CDER Reviews Applications 21-797, 21-798

O-sulfation and *O*-glucuronidation of morphine



Morphine

SULT enzymes in human brain –
1A1
1A2
1A3
1A4
2A1
4A1

Parkinson et al.; *Casarett & Doull's Toxicology: The Basic Science of Poisons*, 2018

SULTs in FDA Guidance on in vitro interaction studies

Is the investigational drug a substrate of metabolizing enzymes?

Phase II enzymes including UDP-glucuronosyltransferases and sulfotransferases are to be considered.

General consideration for evaluation of drug candidates SULT victim and perpetrator potential

Are SULTs the main metabolic pathway?

Are one or more SULTs involved? Are they polymorphically expressed?

What is the likelihood of co-administration with other SULT inhibitors?

Are sulfonate conjugates pharmacologically active?

Are sulfonate conjugates chemically reactive?

Choosing the appropriate in vitro test system

Look at the structure

Functional group	CYP?	Possible non-CYP enzymes	Test systems
Aliphatic alcohol	Yes	ADHs, UGTs, SULTs	HLM, S9, cytosol, hepatocytes
Aliphatic amine	Yes	FMOs, MAOs, UGTs, SULTs, MTs, NATs, peroxidases	HLM, S9, mitochondria, cytosol, hepatocytes
Aniline	Yes	UGTs, SULTs, NATs, peroxidases	HLM, S9, cytosol, mitochondria, hepatocytes
Phenol	Yes	UGTs, SULTs, MTs	HLM, S9, cytosol, hepatocytes, blood

In these test systems other Phase II enzymes capable of metabolizing SULT substrates and inhibitors are present, prominently UGTs.

Parkinson et al.; *Casarett & Doull's Toxicology: The Basic Science of Poisons*, 2018

Choosing the appropriate in vitro test system

Human enzyme	Polymorphic?
SULT1A1	*1 - *4
SULT1A2	*1 - *6
SULT1A3	*1 - *4
SULT1B1	
SULT1C2	*1 - *5
SULT1C3	
SULT1C4	
SULT1D1	
SULT1E1	
SULT2A1	*1 - *3
SULT2B1	
SULT4A1	
SULT6B1	

Genetic polymorphism

- From a practical pharmacogenomics perspective, the most important issue for the SULTs would be the ability to identify low activity alleles that may impair normal drug and hormone metabolism. Allele frequencies for the SULTs do not correlate perfectly with function, but variant alleles with frequencies greater than 5% were uniformly associated with enzyme activities of at least 50% of wild type. More importantly, all the allozymes with enzyme activity less than half of the WT allele had allele frequencies of less than 2.5% in our combined sample.

SULT1C2*5	AA frequency 0.0	CA frequency 0.006	Activity, %WT 0	Protein, %WT 0

Hildebrandt et al., *The Pharmacogenomics Journal* (2007) 7, 133–143

SULT inhibition

Reaction phenotyping

Selected prominent inhibitors of cytosolic sulfotransferases	
Quercetin	17α-Ethynylestradiol
Cyanidin 3-rutinoside	Mefenamic acid
40-Hydroxy-3,30,4,50-tetrachlorobiphenyl	4-Hydroxy-3-methoxymethamphetamine.
Triclosan	Celecoxib

	Standard substrate
SULT1A1	4-nitrophenol
SULT1A3	Dopamine
SULT1E1	17β-Estradiol (low nM)
SULT2A1	Dehydroepiandrosterone

James et al., *Drug Metab Rev*, 2013; 45(4): 401–414

SULT induction

Nuclear receptor	SULT	Receptor activators
Constitutive androstane receptor, CAR	1A1	Phenobarbital, CITCO, TCPOBOP
Pregnane X receptor, PXR	2A1	Rifampin, hyperforin, PCBs
PPARα	2A1	Fibrates
FXR	2A1	Bile acids
Vitamin D receptor, VDR	2A1	Vitamin D_3

SULTs are refractory or only marginally responsive to the enzyme inducing effects of 3-methylcholanthrene (AhR) or phenobarbital (CAR). Induction of SULT1E1 and possibly other SULT enzymes by rifampin may be of clinical importance for oral contraceptives containing ethinyl estradiol.

SULT1E1 in Drug Interactions Involving 17α-Ethinylestradiol

- For drug-drug interactions involving estrogen (17α-ethinylestradiol, EE)-containing oral contraceptives (OC) sponsors of new molecular entities often conduct clinical studies focused on OC as victims of CYP3A induction and inhibition;
- OC such as EE are also metabolized by sulfotransferase 1E1 and UDP-glucuronosyltransferase UGT1A1, expressed in the gut and liver;
- EE-containing OC can induce (e.g., UGT1A4 and CYP2A6) and inhibit (CYP1A2 ≥ CYP2C19 > CYP3A4/5 > CYP2C8, CYP2B6, CYP2D6 and CYP2C9) various CYP forms;
- It is hypothesized that EE differentially modulates CYP expression via potent agonism of the estrogen receptor expressed in the gut and liver

D. Rodrigues, *Clin Pharmacol Ther.* 2021 Aug 2

Considerations for assessing DDI involving EE-containing OC and NME

EE-containing OC as a victim of DDI	
Considerations/Questions	Possible outcomes
Induction Is NME a PXR agonist and a clinically relevant CYP3A inducer? Is NME a clinically relevant inducer of UGT1A1 and/or SULTs (e.g., non-PXR mechanism)?	Breakthrough bleeding (e.g., AUCR < 0.5)
Inhibition Is NME a clinically relevant CYP3A inhibitor? Is NME a clinically relevant inhibitor of SULT1E1, other SULTs, and/or UGT1A1?	EE-induced CV side effects (e.g., AUCR and Cmax > 1.5)

D. Rodrigues, *Clin Pharmacol Ther.* 2021 Aug 2

Considerations for assessing DDI involving EE-containing OC and NME

EE-containing OC as a perpetrator of DDI	
Considerations/Questions	Possible outcomes
Induction	
Is NME metabolized by UGT1A4 or CYP2A6?	Impacts NME PK
Is NME metabolized to an active metabolite that is conjugated by UGT1A4?	Impacts metabolite PK
Inhibition	
Is NME metabolized by CYP1A2 or CYP2C19 (fm > 0.5) with lower oral bioavailability?	Impacts NME C_{max}
Is NME metabolized by CYP1A2 and/or CYP2C19 (fm > 0.5) but oral bioavailability >50%?	Impacts NME $t_{1/2}$, Cl_{sys}
Does the NME have a narrow therapeutic index and present non-linear PK?	Impacts safety profiles

D. Rodrigues, *Clin Pharmacol Ther.* 2021 Aug 2

Interplay of CAR and ERα in regulation of SULT1E1

- SULT1E1, alongside CYP3A4 (>CYP2C9) and UGT1A1, metabolizes estrogen (17α-ethinylestradiol, EE) and may contribute to DDIs involving OC.
- SULT1E1 is induced by phenobarbital treatment or spontaneously in diabetic livers *via* nuclear receptors in mice.
- Constitutive androstane receptor, following its activation by phenobarbital, binds and recruits estrogen receptor α onto the SULT1E1 promoter for subsequent phosphorylation at Ser216 and increased gene transcription.
- Although the FDA Guidance on in vitro drug interactions doesn't discuss induction of SULT enzymes, this process may be an important consideration in development of drugs co-administered with oral contraceptives.
- Estrogen, administered at low doses (~30 mg), has a C_{max} of ~ 0.2 nM, indicating that it can be a good substrate for high specificity, low capacity enzymes, such as the SULTs.
- Human SULT1E1 is induced by CITCO in hepatocytes > resveratrol in HepG2.

Clinical Drug Interaction Studies With Combined Oral Contraceptives, FDA, 2020

Yi et al., *Sci Rep* **10,** 5001 (2020)

Membrane-bound SULTs in Golgi apparatus

- Responsible for sulfonation of glycosaminoglycans, proteins and peptides (potential biotransformation of future drug modalities);
- Five *N*-acetylglucosamine 6-*O*-sulfortansferases have been identified in humans. Defective sulfonation of glycosaminoglycans and proteoglycans such as heparin and chondroitin, which are important components of cartilage, was observed in brachymorpic mice which have a global defect in sulfonation. The sulfonation precedes extension of the glycosaminoglycan chain.
- Heparan sulfate enzyme, HS6OST1, determines distribution of negative charges on the molecule which in turn defines interaction of this glycosaminoglycan with proteins.

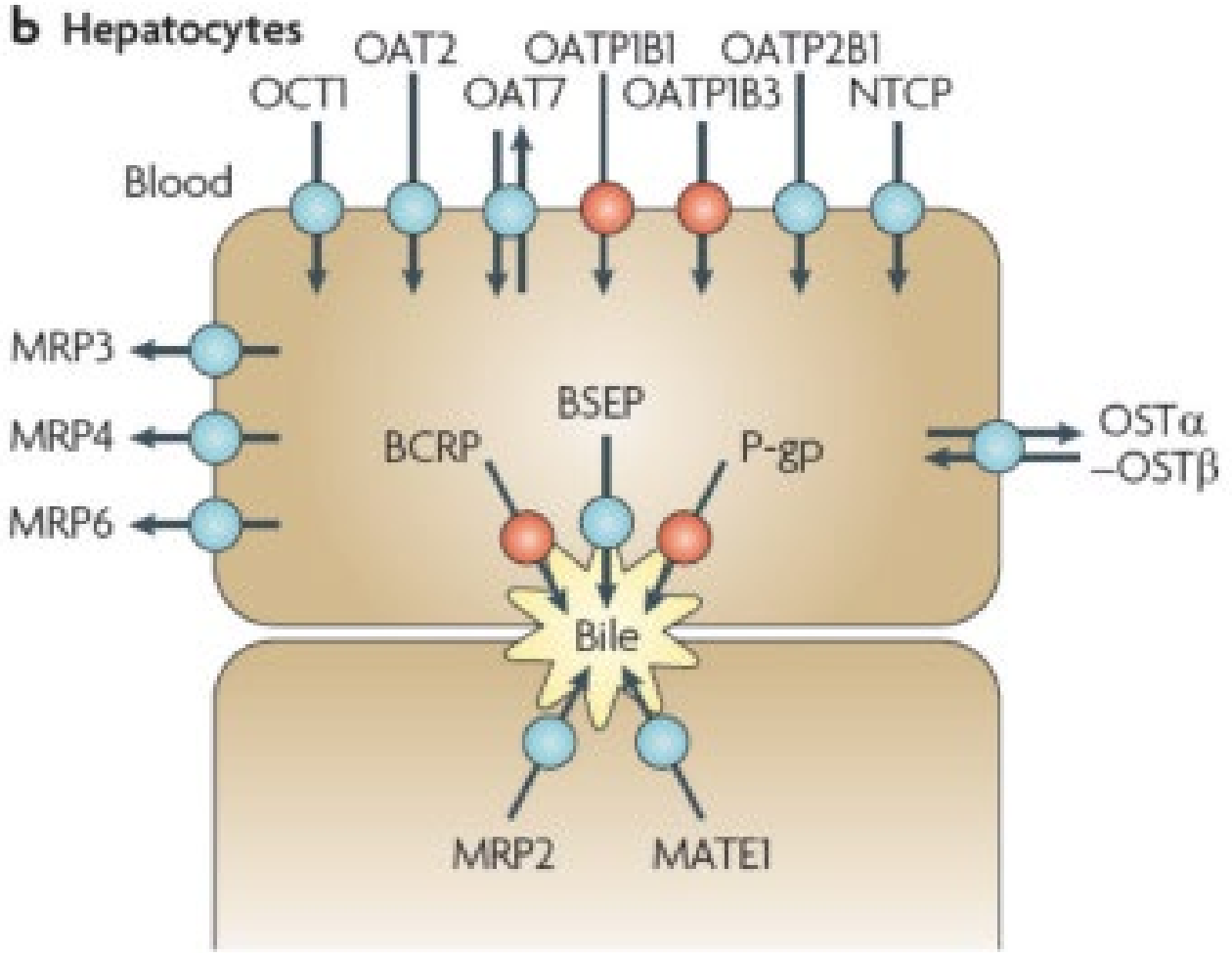
El Masri et al., *Glycoconj J* 34(3), 2017

Transporters in disposition of sulfated drugs

Drug conjugate	Uptake transporters	Efflux transporters
Brexanolone-S	NTCP	
Cabozantinib M2a (sulfate)	OAT3, OATP1B1, OATP1B3	MRP2
Edaravone-S	OAT1, OAT3	BCRP
Ethinylestradiol-3-S	OAT3, OAT4 OATP1B1, OATP2B1	BCRP
6-Hydroxymelatonin-S	OAT3	
Morinidazole-S	OAT1, OAT3	
Relebactam (sulfate)	OAT3, OAT4	MATE1, MATE2K
Thyroxine-S Triiodothyronine-S	NTCP, OATP1B1	
Troglitazone-S	OATP1B1, OATP1B3	BCRP

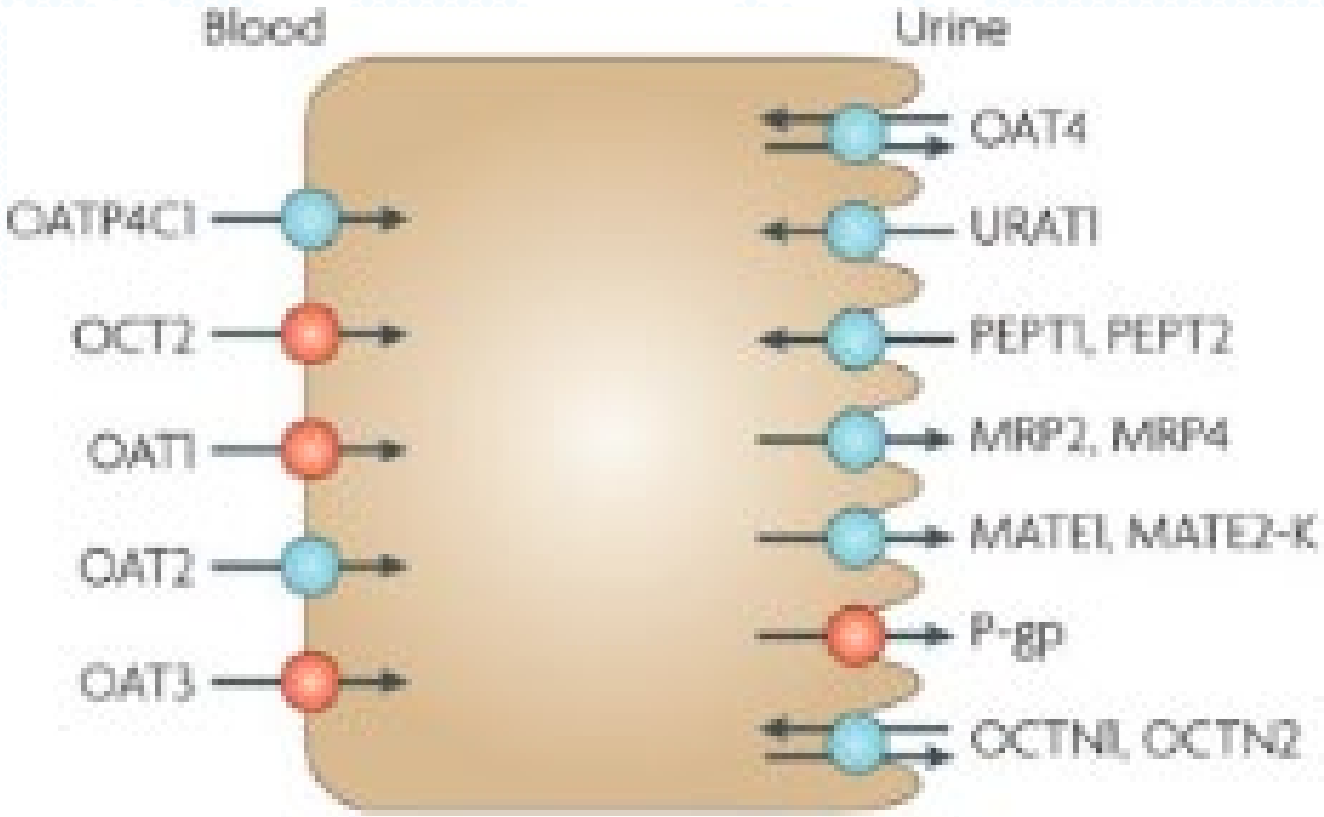
Järvinen et al., *Front Pharmacol*. (2022) **12**, 1-31

Transporters in disposition of sulfated drugs



Järvinen et al., *Front Pharmacol*. (2022) **12**, 1-31

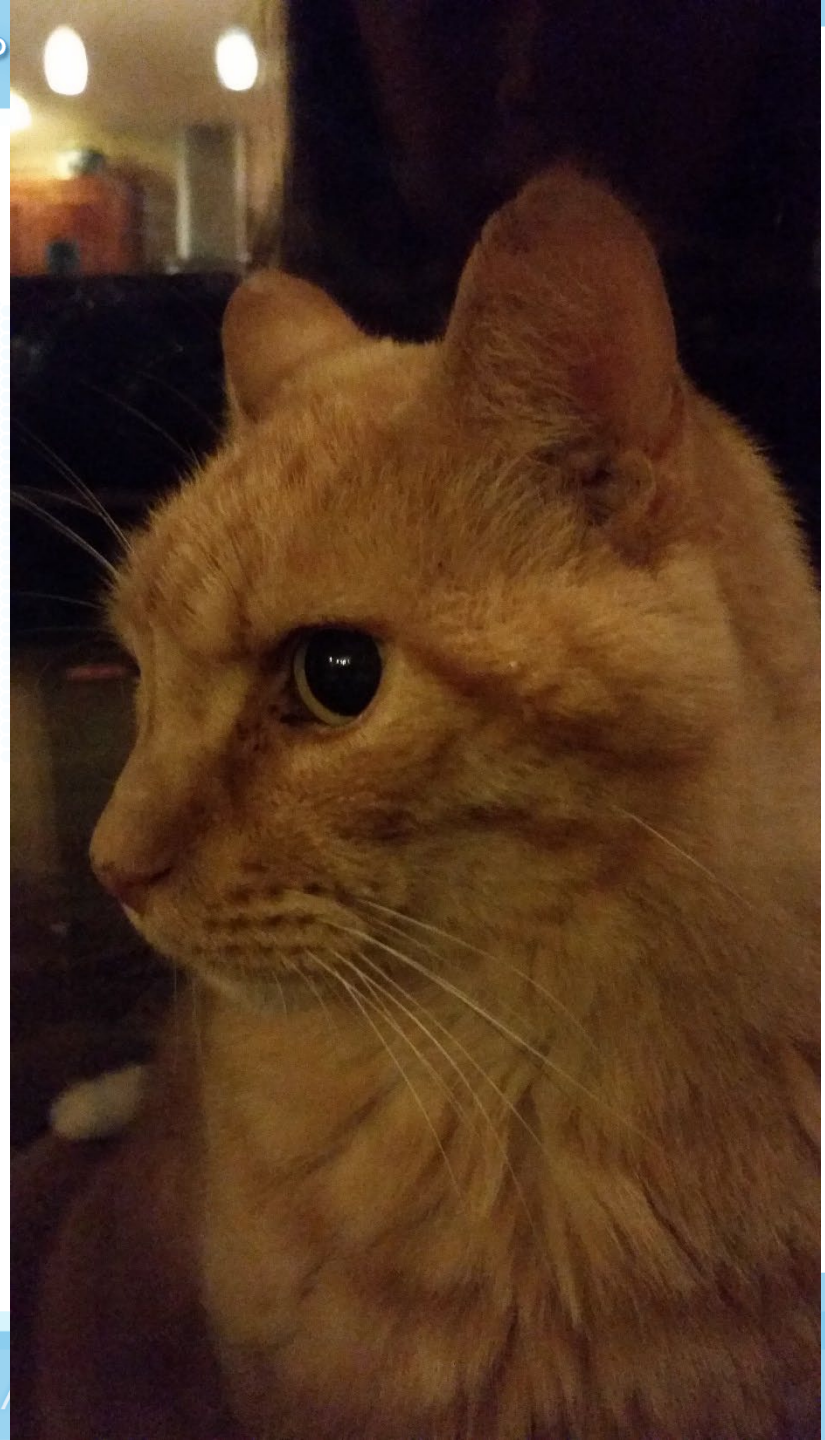
Transporters in disposition of sulfated drugs



Proximal tubule cells

Järvinen et al., *Front Pharmacol*. (2022) **12**, 1-31

Dogs are good glucuronidators, but cats are better sulfonators than dogs.



Abbreviations

ADH, Alcohol dehydrogenase
AhR, Aryl hydrocarbon receptor
ALDH, Aldehyde dehydrogenase
AO, Aldehyde oxidase
APS, Adenosine phosphosulfate
ATP, Adenosine triphosphate
BCRP, Breast cancer resistance protein
CAR, Constitutive androstane receptor
CES, Carboxyl esterase
CITCO, 6-(4-Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde O-(3,4-dichlorobenzyl)oxime
EE, 17α-Ethinylestradiol
FMO, Flavin monooxygenase
FXR, Farensoid X receptor
GST, Glutathione S-transferase
HLM, Human liver microsomes
MAO, Monoamine oxidase
MATE, Multidrug and toxin extrusion transporter
MRP, Multidrug resistance protein
MT, Methyl transferase
NME, New molecular entity
NTCP, sodium/taurocholate co-transporting polypeptide
OAT, Organic anion transporters
OATP, Organic anion transporting polypeptide
OC, Oral contraceptive
PAP, 3'-Phosphoadenosine 5'-phosphate
PAPS, 3'-phosphoadenosine-5'-phosphosulfate
PCB, Polychlorinated biphenyl
PPAR, Peroxisome proliferator-activated receptor
PXR, Pregnane X receptor
SULT, Sulfotransferase
TCPOBOP, 1,4-Bis(3,5-Dichloro-2-pyridinyloxy)benzene
UGT, UDP-glucuronosyltransferase
VDR, Vitamin D receptor
WT, Wild type
XO, Xanthine oxidoreductase

Thank you!